

91200-889617-005610PC_SequenceListing.TXT
SEQUENCE LISTING

<110> Thanos, Christopher D.
Murray, Christopher J.
Yang, Junhao
Stephenson, Heather
Sutro Biopharma, Inc.

<120> Proteolytic Inactivation of Select Proteins
in Bacterial Extracts for Improved Expression

<130> 91200-889617

<140> WO Not yet assigned

<141> Not yet assigned

<150> US 61/713,245

<151> 2012-10-12

<160> 172

<170> FastSEQ for windows Version 4.0

<210> 1

<211> 360

<212> PRT

<213> Escherichia coli

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<223> Release Factor 1 (RF1)

<400> 1

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Met Lys Pro Ser Ile Val Ala Lys Leu Glu Ala Leu His Glu Arg His
 1      5      10      15
Glu Glu Val Gln Ala Leu Leu Gly Asp Ala Gln Thr Ile Ala Asp Gln
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Glu Arg Phe Arg Ala Leu Ser Arg Glu Tyr Ala Gln Leu Ser Asp Val
 35      40      45
Ser Arg Cys Phe Thr Asp Trp Gln Gln Val Gln Glu Asp Ile Glu Thr
 50      55      60
Ala Gln Met Met Leu Asp Asp Pro Glu Met Arg Glu Met Ala Gln Asp
 65      70      75      80
Glu Leu Arg Glu Ala Lys Glu Lys Ser Glu Gln Leu Glu Gln Gln Leu
 85      90      95
Gln Val Leu Leu Leu Pro Lys Asp Pro Asp Asp Glu Arg Asn Ala Phe
100      105      110
Leu Glu Val Arg Ala Gly Thr Gly Gly Asp Glu Ala Ala Leu Phe Ala
115      120      125
Gly Asp Leu Phe Arg Met Tyr Ser Arg Tyr Ala Glu Ala Arg Arg Trp
130      135      140
Arg Val Glu Ile Met Ser Ala Ser Glu Gly Glu His Gly Gly Tyr Lys
145      150      155      160
Glu Ile Ile Ala Lys Ile Ser Gly Asp Gly Val Tyr Gly Arg Leu Lys
165      170      175
Phe Glu Ser Gly Gly His Arg Val Gln Arg Val Pro Ala Thr Glu Ser
180      185      190
Gln Gly Arg Ile His Thr Ser Ala Cys Thr Val Ala Val Met Pro Glu
195      200      205
Leu Pro Asp Ala Glu Leu Pro Asp Ile Asn Pro Ala Asp Leu Arg Ile
210      215      220
Asp Thr Phe Arg Ser Ser Gly Ala Gly Gly Gln His Val Asn Thr Thr
225      230      235      240
Asp Ser Ala Ile Arg Ile Thr His Leu Pro Thr Gly Ile Val Val Glu
245      250      255
Cys Gln Asp Glu Arg Ser Gln His Lys Asn Lys Ala Lys Ala Leu Ser
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Val Leu Gly Ala Arg Ile His Ala Ala Glu Met Ala Lys Arg Gln Gln

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Ala Glu Ala Ser Thr Arg Arg Asn Leu Leu Gly Ser Gly Asp Arg Ser
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Asp Arg Asn Arg Thr Tyr Asn Phe Pro Gln Gly Arg Val Thr Asp His
305   310   315
Arg Ile Asn Leu Thr Leu Tyr Arg Leu Asp Glu Val Met Glu Gly Lys
      325      330      335
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Leu Ala Ala Leu Ser Glu Gln Glu
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<210> 2
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<220>
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Arg Leu Glu Glu Val Asn Ala Glu Leu Glu Gln Pro Asp Val Trp Asn
      35      40      45
Glu Pro Glu Arg Ala Gln Ala Leu Gly Lys Glu Arg Ser Ser Leu Glu
      50      55      60
Ala Val Val Asp Thr Leu Asp Gln Met Lys Gln Gly Leu Glu Asp Val
65      70      75      80
Ser Gly Leu Leu Glu Leu Ala Val Glu Ala Asp Asp Glu Glu Thr Phe
      85      90      95
Asn Glu Ala Val Ala Glu Leu Asp Ala Leu Glu Glu Lys Leu Ala Gln
      100      105      110
Leu Glu Phe Arg Arg Met Phe Ser Gly Glu Tyr Asp Ser Ala Asp Cys
      115      120      125
Tyr Leu Asp Ile Gln Ala Gly Ser Gly Gly Thr Glu Ala Gln Asp Trp
      130      135      140
Ala Ser Met Leu Glu Arg Met Tyr Leu Arg Trp Ala Glu Ser Arg Gly
145      150      155      160
Phe Lys Thr Glu Ile Ile Glu Glu Ser Glu Gly Glu Val Ala Gly Ile
      165      170      175
Lys Ser Val Thr Ile Lys Ile Ser Gly Asp Tyr Ala Tyr Gly Trp Leu
      180      185      190
Arg Thr Glu Thr Gly Val His Arg Leu Val Arg Lys Ser Pro Phe Asp
      195      200      205
Ser Gly Gly Arg Arg His Thr Ser Phe Ser Ser Ala Phe Val Tyr Pro
      210      215      220
Glu Val Asp Asp Asp Ile Asp Ile Glu Ile Asn Pro Ala Asp Leu Arg
225      230      235      240
Ile Asp Val Tyr Arg Thr Ser Gly Ala Gly Gly Gln His Val Asn Arg
      245      250      255
Thr Glu Ser Ala Val Arg Ile Thr His Ile Pro Thr Gly Ile Val Thr
      260      265      270
Gln Cys Gln Asn Asp Arg Ser Gln His Lys Asn Lys Asp Gln Ala Met
      275      280      285
Lys Gln Met Lys Ala Lys Leu Tyr Glu Leu Glu Met Gln Lys Lys Asn
      290      295      300
Ala Glu Lys Gln Ala Met Glu Asp Asn Lys Ser Asp Ile Gly Trp Gly
305      310      315      320
Ser Gln Ile Arg Ser Tyr Val Leu Asp Asp Ser Arg Ile Lys Asp Leu
      325      330      335
Arg Thr Gly Val Glu Thr Arg Asn Thr Gln Ala Val Leu Asp Gly Ser
      340      345      350
Leu Asp Gln Phe Ile Glu Ala Ser Leu Lys Ala Gly Leu
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<210> 3
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 <213> Escherichia coli

<220>
 <223> Outer Membrane Protein T1 (OmpT1)

<220>
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 1 10
 Asn Ala Asp Ile Ser Leu Gly Thr Leu Ser Gly Lys Thr Lys Glu Arg
 15 20 25
 Val Tyr Leu Ala Glu Glu Gly Gly Arg Lys Val Ser Gln Leu Asp Trp
 30 35 40
 Lys Phe Asn Asn Ala Ala Ile Ile Lys Gly Ala Ile Asn Trp Asp Leu
 45 50 55 60
 Met Pro Gln Ile Ser Ile Gly Ala Ala Gly Trp Thr Thr Leu Gly Ser
 65 70 75
 Arg Gly Gly Asn Met Val Asp Gln Asp Trp Met Asp Ser Ser Asn Pro
 80 85 90
 Gly Thr Trp Thr Asp Glu Ser Arg His Pro Asp Thr Gln Leu Asn Tyr
 95 100 105
 Ala Asn Glu Phe Asp Leu Asn Ile Lys Gly Trp Leu Leu Asn Glu Pro
 110 115 120
 Asn Tyr Arg Leu Gly Leu Met Ala Gly Tyr Gln Glu Ser Arg Tyr Ser
 125 130 135 140
 Phe Thr Ala Arg Gly Gly Ser Tyr Ile Tyr Ser Ser Glu Glu Gly Phe
 145 150 155
 Arg Asp Asp Ile Gly Ser Phe Pro Asn Gly Glu Arg Ala Ile Gly Tyr
 160 165 170
 Lys Gln Arg Phe Lys Met Pro Tyr Ile Gly Leu Thr Gly Ser Tyr Arg
 175 180 185
 Tyr Glu Asp Phe Glu Leu Gly Gly Thr Phe Lys Tyr Ser Gly Trp Val
 190 195 200
 Glu Ser Ser Asp Asn Asp Glu His Tyr Asp Pro Gly Lys Arg Ile Thr
 205 210 215 220
 Tyr Arg Ser Lys Val Lys Asp Gln Asn Tyr Tyr Ser Val Ala Val Asn
 225 230 235
 Ala Gly Tyr Tyr Val Thr Pro Asn Ala Lys Val Tyr Val Glu Gly Ala
 240 245 250
 Trp Asn Arg Val Thr Asn Lys Lys Gly Asn Thr Ser Leu Tyr Asp His
 255 260 265
 Asn Asn Asn Thr Ser Asp Tyr Ser Lys Asn Gly Ala Gly Ile Glu Asn
 270 275 280
 Tyr Asn Phe Ile Thr Thr Ala Gly Leu Lys Tyr Thr Phe
 285 290 295

<210> 4
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> synthetic switch loop region of Release Factor 1
 (RF1), amino acids 287 to 304

<400> 4

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Gln Gln Ala Glu Ala Ser Thr Arg Arg Asn Leu Leu Gly Ser Gly Asp
 1 5 10 15
 Arg Ser

<210> 5
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> synthetic N296K mutation in the switch loop region
 of Release Factor 1 (RF1), amino acids 287 to 304

<400> 5
 Gln Gln Ala Glu Ala Ser Thr Arg Arg Lys Leu Leu Gly Ser Gly Asp
 1 5 10 15
 Arg Ser

<210> 6
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> synthetic N296R mutation in the switch loop region
 of Release Factor 1 (RF1), amino acids 287 to 304

<400> 6
 Gln Gln Ala Glu Ala Ser Thr Arg Arg Arg Leu Leu Gly Ser Gly Asp
 1 5 10 15
 Arg Ser

<210> 7
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
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 of Release Factor 1 (RF1), amino acids 287 to 304

<400> 7
 Gln Gln Ala Glu Ala Ser Thr Arg Arg Asn Lys Leu Gly Ser Gly Asp
 1 5 10 15
 Arg Ser

<210> 8
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> synthetic L297R mutation in the switch loop region
 of Release Factor 1 (RF1), amino acids 287 to 304

<400> 8
 Gln Gln Ala Glu Ala Ser Thr Arg Arg Asn Arg Leu Gly Ser Gly Asp
 1 5 10 15
 Arg Ser

<210> 9
 <211> 18
 <212> PRT

<213> Artificial Sequence

<220>

<223> synthetic L297V mutation in the switch loop region
of Release Factor 1 (RF1), amino acids 287 to 304

<400> 9

Gln Gln Ala Glu Ala Ser Thr Arg Arg Asn Val Leu Gly Ser Gly Asp
1 5 10 15
Arg Ser

<210> 10

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic L298K mutation in the switch loop region
of Release Factor 1 (RF1), amino acids 287 to 304

<400> 10

Gln Gln Ala Glu Ala Ser Thr Arg Arg Asn Leu Lys Gly Ser Gly Asp
1 5 10 15
Arg Ser

<210> 11

<211> 18

<212> PRT

<213> Artificial Sequence

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of Release Factor 1 (RF1), amino acids 287 to 304

<400> 11

Gln Gln Ala Glu Ala Ser Thr Arg Arg Asn Leu Arg Gly Ser Gly Asp
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Arg Ser

<210> 12

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<213> Artificial Sequence

<220>

<223> synthetic N296K,L297K mutation in the switch loop region
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<400> 12

Gln Gln Ala Glu Ala Ser Thr Arg Arg Lys Lys Leu Gly Ser Gly Asp
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<210> 13

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

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region of Release Factor 1 (RF1), amino acids 287 to 304

<400> 13

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Arg Ser

<210> 14
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<212> PRT
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<220>
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<210> 15
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<220>
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of Release Factor 1 (RF1), amino acids 287 to 304

<400> 15
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<210> 16
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<220>
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of Release Factor 1 (RF1), amino acids 287 to 304

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<210> 17
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<220>
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Gln Gln Ala Glu Ala Ser Thr Arg Arg Arg Val Leu Gly Ser Gly Asp
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<210> 18
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<220>

<223> synthetic N296K,L297K,L298K mutation in the switch loop region of Release Factor 1 (RF1), amino acids 287 to 304

<400> 18

Gln Gln Ala Glu Ala Ser Thr Arg Arg Lys Lys Lys Gly Ser Gly Asp
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Arg Ser

<210> 19

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic N296K,L297K,L298R mutation in the switch loop region of Release Factor 1 (RF1), amino acids 287 to 304

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Arg Ser

<210> 20

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic N296K,L297R,L298K = A17 mutation in the switch loop region of Release Factor 1 (RF1), amino acids 287 to 304

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1 5 10 15
Arg Ser

<210> 21

<211> 18

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<223> synthetic N296K,L297R,L298R = A18 mutation in the switch loop region of Release Factor 1 (RF1), amino acids 287 to 304

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Arg Ser

<210> 22

<211> 18

<212> PRT

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<220>

<223> synthetic N296R,L297R,L298R mutation in the switch loop region of Release Factor 1 (RF1), amino acids 287 to 304

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1 5 10 15

Arg Ser

<210> 23
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<220>
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<210> 24
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 1 5 10 15
 Arg Ser

<210> 25
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<220>
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 1 5 10 15
 Arg Ser

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<400> 26
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 1 5 10 15
 Arg Ser

<210> 27
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 <213> Artificial Sequence

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<220>
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 (RF1), amino acids 287 to 304
 <400> 27
 Gln Gln Ala Arg Arg Gly Thr Arg Arg Asn Leu Leu Gly Ser Gly Asp
 1 5 10 15
 Arg Ser

<210> 28
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
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 <400> 28
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 1 5 10 15
 Arg Ser

<210> 29
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> synthetic OmpT cleavage peptide sequence inserted
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 (RF1), amino acids 287 to 304
 <400> 29
 Gln Gln Ala Glu Ala Arg Arg Gly Arg Asn Leu Leu Gly Ser Gly Asp
 1 5 10 15
 Arg Ser

<210> 30
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
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 in the switch loop region of Release Factor 1
 (RF1), amino acids 287 to 304
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 1 5 10 15
 Arg Ser

<210> 31
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
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1 5 10 15
Arg Ser

<210> 32
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<212> PRT
<213> Artificial Sequence

<220>
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(RF1), amino acids 287 to 304

<400> 32
Gln Gln Ala Glu Ala Ser Thr Ala Arg Arg Gly Leu Gly Ser Gly Asp
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Arg Ser

<210> 33
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic OmpT cleavage peptide sequence inserted
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Arg Ser

<210> 34
<211> 18
<212> PRT
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<220>
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(RF1), amino acids 287 to 304

<400> 34
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<210> 35
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<213> Artificial Sequence

<220>
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(RF1), amino acids 287 to 304

<400> 35
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 Arg Ser 5 10 15

<210> 36
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 <212> PRT
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<220>
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 1 5 10 15
 Arg Ser

<210> 37
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 <213> Artificial Sequence

<220>
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 (RF1), amino acids 287 to 304

<400> 37
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 1 5 10 15
 Arg Ser

<210> 38
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
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<210> 39
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
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<400> 39
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<210> 40
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 <213> Artificial Sequence

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 1 5 10 15
 Arg Arg

<210> 41
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 <213> Artificial Sequence

 <220>
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 1 5 10 15
 Arg Ser

<210> 42
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 <213> Artificial Sequence

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 <223> synthetic OmpT cleavage peptide sequence inserted
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 (RF1), amino acids 287 to 304

 <400> 42
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 1 5 10 15
 Arg Ser

<210> 43
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 <220>
 <223> synthetic OmpT cleavage peptide sequence inserted
 in the switch loop region of Release Factor 1
 (RF1), amino acids 287 to 304

 <400> 43
 Gln Gln Ala Glu Ala Trp Leu Ala Ala Arg Arg Gly Arg Gly Gly Asp
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<210> 44
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<220>
<223> synthetic OmpT cleavage peptide sequence inserted
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Arg Ser

<210> 45
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<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic OmpT cleavage peptide sequence inserted
      in the switch loop region of Release Factor 1
      (RF1), amino acids 287 to 304

<400> 45
Gln Gln Ala Trp Gly Gly Arg Trp Ala Arg Lys Lys Gly Thr Ile Asp
 1           5           10           15
Arg Ser

<210> 46
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
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      in the switch loop region of Release Factor 1
      (RF1), amino acids 287 to 304

<400> 46
Gln Gln Ala Glu Trp Gly Gly Arg Trp Ala Arg Lys Lys Gly Thr Ile
 1           5           10           15
Arg Ser

<210> 47
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<220>
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<400> 47
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<210> 48
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<220>
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<210> 49
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<220>
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<400> 49
Trp Gly Gly Arg Trp Ala Arg Lys Lys Gly Thr Ile
 1             5             10

<210> 50
<211> 9
<212> PRT
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<220>
<223> synthetic C-terminal sequence of short form of
      lambda phage Gam protein (Gams)

<400> 50
Gly Gly Ser His His His His His His
 1             5

<210> 51
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic short form of lambda phage Gam protein
      (Gams) gene amplification primer

<400> 51
atatatcata tgaacgctta ttacattcag gatcgtcttg ag                      42

<210> 52
<211> 72
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic short form of lambda phage Gam protein
      (Gams) gene amplification primer

<400> 52
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aacctggtgg tg                                                         72

<210> 53
<211> 58
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic 5'-fragment including T7 promoter, constant region
      of the N-terminal sequence and the mutation site first step
      PCR amplification oligo primer 5chiT2PT7

<400> 53
gcgtactagc gtaccacgtg gctggtggcc gattcattaa tgcagctggc acgacagg    58

<210> 54

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<211> 60
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> synthetic 3'-fragment including mutation site, constant C-terminal region and T7 terminator sequences first step PCR amplification oligo primer 3chiT2T7

<400> 54
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<210> 55
 <211> 28
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> synthetic 5'-fragment and 3'-fragment assembly overlapping second step PCR single primer 5chiT2

<400> 55
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<210> 56
 <211> 35
 <212> DNA
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<220>
 <223> synthetic WT RF1 variant OmpT cleavage site screening forward primer

<400> 56
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<210> 57
 <211> 35
 <212> DNA
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<220>
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<400> 57
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<210> 58
 <211> 38
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> synthetic E76K RF1 variant OmpT cleavage site screening forward primer

<400> 58
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<210> 59
 <211> 40
 <212> DNA
 <213> Artificial Sequence

<220>
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91200-889617-005610PC_SequenceListing.TXT
screening forward primer

<400> 59
caggatgaac tgcgcaaagc taaagaaaaa agcgagcaac 40

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<211> 40
<212> DNA
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screening forward primer

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<210> 62
<211> 38
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screening forward primer

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gccaaaagat cctgatgacc gtcgtaacgc cttcctcg 38

<210> 63
<211> 34
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screening forward primer

<400> 63
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<210> 64
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screening forward primer

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<210> 65
<211> 36
<212> DNA

<213> Artificial Sequence

<220>
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<400> 65
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<210> 66
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<220>
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<400> 66
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<210> 67
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 site screening forward primer

<400> 67
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<210> 68
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 site screening forward primer

<400> 68
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<210> 69
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<210> 71
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<210> 75
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screening reverse primer

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screening reverse primer

<400> 82
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<210> 83
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screening reverse primer

<400> 83
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<210> 84
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site screening reverse primer

<400> 84
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<210> 85
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<220>
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site screening reverse primer

<400> 85
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site screening reverse primer

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site screening reverse primer

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<210> 88
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<210> 89
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<210> 91
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 acttacaact tcccg 75

<210> 93
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 <212> DNA
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insertion forward oligo primer

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acttacaact tcccg 75

<210> 94
<211> 75
<212> DNA
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<220>
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insertion forward oligo primer

<400> 94
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acttacaact tcccg 75

<210> 95
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insertion forward oligo primer

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acttacaact tcccg 75

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insertion forward oligo primer

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acttacaact tcccg 75

<210> 97
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acttacaact tcccg 75

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insertion forward oligo primer

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acttacaact tcccg 75

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<400> 99
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acttacaact tcccg 75

<210> 100
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<220>
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insertion forward oligo primer

<400> 100
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acttacaact tcccg 75

<210> 101
<211> 75
<212> DNA
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<220>
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insertion forward oligo primer

<400> 101
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acttacaact tcccg 75

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insertion forward oligo primer

<400> 102
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acttacaact tcccg 75

<210> 103
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<220>
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insertion forward oligo primer

<400> 103
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acttacaact tcccg 75

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acttacaact tcccg                                         75

<210> 105
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<212> DNA
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<400> 105
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acttacaact tcccg                                         75

<210> 106
<211> 75
<212> DNA
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<220>
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<400> 106
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acttacaact tcccg                                         75

<210> 107
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<220>
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<400> 107
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acttacaact tcccg                                         75

<210> 108
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<212> DNA
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<220>
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<400> 108
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acttacaact tcccg                                         75

<210> 109
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<220>
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acttacaact tcccg                                         75

<210> 110
<211> 75
<212> DNA
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<220>
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      insertion forward oligo primer

<400> 110
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acttacaact tcccg                                         75

<210> 111
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<220>
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      insertion forward oligo primer

<400> 111
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acttacaact tcccg                                         75

<210> 112
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<212> DNA
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<220>
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acttacaact tcccg                                         75

<210> 113
<211> 75
<212> DNA
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<220>
<223> synthetic No. 21 RF1 variant OmpT cleavage peptide
      insertion forward oligo primer

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acttacaact tcccg                                         75

<210> 114
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<220>
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      insertion forward oligo primer

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acttacaact tcccg                                         75

<210> 115
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic No. 0 RF1 variant OmpT cleavage peptide
      insertion reverse oligo primer

<400> 115
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tgccatttca gcagc                                         75

<210> 116
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic No. 1 RF1 variant OmpT cleavage peptide
      insertion reverse oligo primer

<400> 116
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tgccatttca gcagc                                         75

<210> 117
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic No. 2 RF1 variant OmpT cleavage peptide
      insertion reverse oligo primer

<400> 117
gctgcgatcg ccactcccca gcaggttacg acgggtacca cggcgtgcct gttggcgttt 60
tgccatttca gcagc                                         75

<210> 118
<211> 75
<212> DNA
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<220>
<223> synthetic No. 3 RF1 variant OmpT cleavage peptide
      insertion reverse oligo primer

<400> 118
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tgccatttca gcagc                                         75

<210> 119
<211> 75
<212> DNA
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<220>
<223> synthetic No. 4 RF1 variant OmpT cleavage peptide
      insertion reverse oligo primer

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tgccatttca gcagc 75

<210> 120
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<220>
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<400> 120
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tgccatttca gcagc 75

<210> 121
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<220>
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insertion reverse oligo primer

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tgccatttca gcagc 75

<210> 122
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<212> DNA
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<220>
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insertion reverse oligo primer

<400> 122
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tgccatttca gcagc 75

<210> 123
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<220>
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insertion reverse oligo primer

<400> 123
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tgccatttca gcagc 75

<210> 124
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<212> DNA
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<220>
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insertion reverse oligo primer

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tgccatttca gcagc

75

<210> 125

<211> 75

<212> DNA

<213> Artificial Sequence

<220>

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<400> 125

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tgccatttca gcagc 75

<210> 126

<211> 75

<212> DNA

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insertion reverse oligo primer

<400> 126

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tgccatttca gcagc 75

<210> 127

<211> 75

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic No. 12 RF1 variant OmpT cleavage peptide
insertion reverse oligo primer

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tgccatttca gcagc 75

<210> 128

<211> 75

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic No. 13 RF1 variant OmpT cleavage peptide
insertion reverse oligo primer

<400> 128

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tgccatttca gcagc 75

<210> 129

<211> 75

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic No. 14 RF1 variant OmpT cleavage peptide
insertion reverse oligo primer

<400> 129

accacggcgt gcactccccca gcagggttacg acgggtagac gcttcggcct gttggcgttt 60
tgccatttca gcagc 75

<210> 130

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<211> 75
<212> DNA
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<220>
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tgccatttca gcagc                                                    75

<210> 131
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
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      insertion reverse oligo primer

<400> 131
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tgccatttca gcagc                                                    75

<210> 132
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic No. 17 RF1 variant OmpT cleavage peptide
      insertion reverse oligo primer

<400> 132
gctgcgatcg ccactcccg caccgacgcg acgcgctgcc agccaggcct gttggcgttt 60
tgccatttca gcagc                                                    75

<210> 133
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic No. 18 RF1 variant OmpT cleavage peptide
      insertion reverse oligo primer

<400> 133
gctgcgatcg ccactgccac gaccgcgacg cgctgccagc cattcggcct gttggcgttt 60
tgccatttca gcagc                                                    75

<210> 134
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic No. 19 RF1 variant OmpT cleavage peptide
      insertion reverse oligo primer

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tgccatttca gcagc                                                    75

<210> 135
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<212> DNA
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<220>
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tgccatttca gcagc 75

<210> 136
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic No. 21 RF1 variant OmpT cleavage peptide
      insertion reverse oligo primer

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tgccatttca gcagc 75

<210> 137
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic No. 22 RF1 variant OmpT cleavage peptide
      insertion reverse oligo primer

<400> 137
gctgcgaata gtacctttct tgcgagccca acggccaccc cattcggcct gttggcgttt 60
tgccatttca gcagc 75

<210> 138
<211> 66
<212> DNA
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<220>
<223> synthetic RF1 amplification primer 5His-RF1

<400> 138
catatgcac accatcacca tcacggtggg ggctctaagc cttctatcgt tgccaaactg 60
gaagcc 66

<210> 139
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic RF1 amplification primer 3RF1

<400> 139
gtcgacttat tcctgctcgg acaacgccgc cag 33

<210> 140
<211> 90
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic oligonucleotide-Mediated Allelic
      Replacement (OMAR) PCR oligo 1opRF1 KR

<400> 140

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gggaagttgt aagtacggtt acggtcgctg cgatcccctg aaccaagacg ctttcgacgg 60
 gtagacgctt cggcctgttg gcgttttgcc 90

<210> 141
 <211> 90
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> synthetic oligonucleotide-Mediated Allelic
 Replacement (OMAR) PCR oligo 1opRF1 KRR

<400> 141
 gggaagttgt aagtacggtt acggtcgctg cgatcccctg aaccacgacg ctttcgacgg 60
 gtagacgctt cggcctgttg gcgttttgcc 90

<210> 142
 <211> 90
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> synthetic oligonucleotide-Mediated Allelic
 Replacement (OMAR) PCR oligo 1opRF1 KRR

<400> 142
 gggaagttgt aagtacggtt acggtcgctg cgatcccctg aacccttacg ctttcgacgg 60
 gtagacgctt cggcctgttg gcgttttgcc 90

<210> 143
 <211> 22
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> synthetic Mismatch Amplification Mutation Assay
 (MAMA) PCR oligo 3KR op-PCR

<400> 143
 gcgatcccct gaaccaagac gc 22

<210> 144
 <211> 21
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> synthetic Mismatch Amplification Mutation Assay
 (MAMA) PCR oligo 3 KRR op-PCR

<400> 144
 cgatcccctg aaccacgacg c 21

<210> 145
 <211> 23
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> synthetic Mismatch Amplification Mutation Assay
 (MAMA) PCR oligo 3KRK op-PCR

<400> 145
 tgcgatcccc tgaaccctta cgc 23

<210> 146
 <211> 22
 <212> DNA

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<213> Artificial Sequence

<220>

<223> synthetic Mismatch Amplification Mutation Assay
(MAMA) PCR oligo 5 RF1 op-PCR

<400> 146
cgtgacgggg ataacgaacg cc 22

<210> 147
<211> 19
<212> DNA
<213> Artificial Sequence

<220>

<223> synthetic rolling circle amplification and primer
extension sequencing primer T7

<400> 147
taatacgact cactatagg 19

<210> 148
<211> 18
<212> DNA
<213> Artificial Sequence

<220>

<223> synthetic rolling circle amplification and primer
extension sequencing primer T7 term

<400> 148
gctagttatt gctcagcg 18

<210> 149
<211> 39
<212> DNA
<213> Artificial Sequence

<220>

<223> synthetic site directed mutagenesis variant
SP-00067_v422 sense oligo primer for introducing
amber codon in Herceptin

<400> 149
cgttggcagc agggtaatta gttcagctgc agcgttatg 39

<210> 150
<211> 39
<212> DNA
<213> Artificial Sequence

<220>

<223> synthetic site directed mutagenesis variant
SP-00067_v422 antisense oligo primer for
introducing amber codon in Herceptin

<400> 150
cataacgctg cagctgaact aattaccctg ctgccaacg 39

<210> 151
<211> 42
<212> DNA
<213> Artificial Sequence

<220>

<223> synthetic site directed mutagenesis variant
SP-00127_s415 sense oligo primer for introducing
amber codon in Herceptin

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<400> 151
gcaagctgac cgtcgataaa tagcgttggc agcagggtaa tg 42

<210> 152
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic site directed mutagenesis variant
SP-00127_S415 antisense oligo primer for
introducing amber codon in Herceptin

<400> 152
cattaccctg ctgccaacgc tatttatcga cggtcagctt gc 42

<210> 153
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic site directed mutagenesis variant
SP-00128_Q418 sense oligo primer for introducing
amber codon in Herceptin

<400> 153
cgataaaagc cgttggttagc aggtaaatgt gttagc 36

<210> 154
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
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SP-00128_Q418 antisense oligo primer for
introducing amber codon in Herceptin

<400> 154
ctgaacacat taccctgcta ccaacggctt ttatcg 36

<210> 155
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic site directed mutagenesis variant
SP-00114_P343 sense oligo primer for introducing
amber codon in Herceptin

<400> 155
gcaaagcgaa aggccaatag cgtgaaccgc aggtc 35

<210> 156
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
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SP-00114_P343 antisense oligo primer for
introducing amber codon in Herceptin

<400> 156
gacctgcggt tcacgctatt ggcctttcgc tttagc 35

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<210> 157
 <211> 40
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> synthetic site directed mutagenesis variant
 SP-00112_G341 sense oligo primer for introducing
 amber codon in Herceptin

 <400> 157
 gacgatcagc aaagcgaaat agcaaccgcg tgaaccgcag 40

 <210> 158
 <211> 40
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> synthetic site directed mutagenesis variant
 SP-00112_G341 antisense oligo primer for
 introducing amber codon in Herceptin

 <400> 158
 ctgcggttca cgcggttgct atttcgcttt gctgacgctc 40

 <210> 159
 <211> 41
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> synthetic site directed mutagenesis variant
 SP-00102_K320 sense oligo primer for introducing
 amber codon in Herceptin

 <400> 159
 gctgaatggt aaagaatact agtgcaaagt gagcaacaag g 41

 <210> 160
 <211> 41
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> synthetic site directed mutagenesis variant
 SP-00102_K320 antisense oligo primer for
 introducing amber codon in Herceptin

 <400> 160
 ccttggtgct cactttgcac tagtattctt taccattcag c 41

 <210> 161
 <211> 39
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> synthetic site directed mutagenesis variant
 SP-00066_F404 sense oligo primer for introducing
 amber codon in Herceptin

 <400> 161
 ctggacagcg acggtagcta gtttctgtat agcaagctg 39

 <210> 162
 <211> 39

<212> DNA
 <213> Artificial Sequence

 <220>
 <223> synthetic site directed mutagenesis variant
 SP-00066_F404 antisense oligo primer for
 introducing amber codon in Herceptin

 <400> 162
 cagcttgcta tacagaaact agctaccgtc gctgtccag 39

 <210> 163
 <211> 32
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> synthetic site directed mutagenesis variant
 SP-00113_Q342 sense oligo primer for introducing
 amber codon in Herceptin

 <400> 163
 gcaaagcgaa aggctagccg cgtgaaccgc ag 32

 <210> 164
 <211> 32
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> synthetic site directed mutagenesis variant
 SP-00113_Q342 antisense oligo primer for
 introducing amber codon in Herceptin

 <400> 164
 ctgcggttca cgcggctagc ctttcgcttt gc 32

 <210> 165
 <211> 42
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> synthetic site directed mutagenesis variant
 SP-00096_T299 sense oligo primer for introducing
 amber codon in Herceptin

 <400> 165
 gtgaggaaca atacaatagc tagtatcgcg tagtgagcgt gc 42

 <210> 166
 <211> 42
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> synthetic site directed mutagenesis variant
 SP-00096_T299 antisense oligo primer for
 introducing amber codon in Herceptin

 <400> 166
 gcacgctcac tacgcgatac tagctattgt attgttcctc ac 42

 <210> 167
 <211> 30
 <212> DNA
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<220>
<223> synthetic site directed mutagenesis variant
      SP-00120_Y373 sense oligo primer for introducing
      amber codon in Herceptin

<400> 167
ggtgaagggc ttttagccga gcgacatcgc
30

<210> 168
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic site directed mutagenesis variant
      SP-00120_Y373 antisense oligo primer for
      introducing amber codon in Herceptin

<400> 168
gcgatgtcgc tcggctaaaa gcccttcacc
30

<210> 169
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic site directed mutagenesis variant
      SP-00094_N297 sense oligo primer for introducing
      amber codon in Herceptin

<400> 169
cgcgtaggagga acaatactag agcacgtatc gcgtagtg
38

<210> 170
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic site directed mutagenesis variant
      SP-00094_N297 antisense oligo primer for
      introducing amber codon in Herceptin

<400> 170
cactacgcga tacgtgctct agtattgttc ctcacgcg
38

<210> 171
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic site directed mutagenesis variant
      SP-00125_F405 sense oligo primer for introducing
      amber codon in Herceptin

<400> 171
gacagcgacg gtagcttcta gctgtatagc aagctgac
38

<210> 172
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic site directed mutagenesis variant
      SP-00125_F405 antisense oligo primer for

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introducing amber codon in Herceptin

<400> 172

gtcagcttgc tatacagcta gaagctaccg tcgctgtc

38